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"Evaluation of pathogenic abilities of certain fungi isolated from common leafy vegetable crops of Chhattisgarh"

Introduction

The science that deals with the diseased plants is known as plant pathology. Plant pathology involves the study of pathogen, identification, disease cycle, etiology, epidemiology, resistance and its effect with overall management of plant diseases. Now disease deals as interaction of 'host pathogen suscepts'. Thus the development of a plant disease depends upon three aspects, a susceptible host, a virulent pathogen and a favorable environment. These aspects in sufficient duration allow infection by pathogen and cause subsequent development of disease in plants.

Leafy vegetables not only make our diet balanced but also act as a natural source of nutrients available in soil. These vegetables do not require specific or extra fertilizers and more over their cultivation procedure is quite simple. Therefore there is a tremendous scope which must be done with scientific skill and approach.

The newly formed Chhattisgarh state has been declared as a herbal state is known for its rich biodiversity, ample number of medicinal plants, plants of ethnobotanical significance and a very large number of foliaceous vegetables crops. The leafy vegetables are popularly called "Bhaaji" and are relished by both urban and rural population. These leafy vegetables are available in different crop seasons and are a rich source of essential minerals.

The scientific value of foliaceous vegetables are under practice among the people of Chhattisgarh state as traditional value from one generation to another generation. The traditional values of some leafy vegetables are as *Cassia tora,* play a significant role in gout and rheumatism, *Ipomoea aquatica* are eaten for constipation and known as tonic for lacting women. *Launaea asplenifolia* is source of vitamin A whereas *Amaranthus tricolor* are rich in iron. Liver problems are solved by eating, *Leucas cephalotes. Rumex vesicaris and Amaranthus hybridis* are famous to cure gynecological troubles. *Raphnus sativus* rich in fibers and solved digestive troubles. *Chenopodium album* is known as a cure agent of leucoderma.

In this piece of research work certain leafy vegetable crops viz: *Cassia tora* (Charota), *Ipomoea aqatica* (Karmatta) , *Launaea asplenifolia* (Kusum), *Amaranthus tricolor* (Lal bhaaji), *Leucas cephlotes* (Gumma), *Rumex vesicaris* (Amari), *Amaranthus hybridus* (Chaulai), *Raphanus sativus* (Muli bhaaji), *Chenopodium album* (Bathua), *Oxalis corniculata* (Tinpania) have been selected for their phytopathological studies on the basis of their ethnobotnical significance. These crops are popular among the people of Chhattisgarh belonging to urban, rural and tribal communities for their traditional values.

A brief review of the work already done in the field

Leafy vegetables have been a topic of interest in our state, India and abroad as because of their nutritive value hardiness and medicinal significance. Reports of pathogens and their pathogenesis on the foliaceous vegetables crops have also been researched all over the world.

The indigenous people have helped in conservation of bio-diversity. Ethnic people needs to be surved for identification of plants associated with various ethno-botanical uses followed by phytochemical studies.

Many of them are resilient, adaptive, and tolerate adverse climatic conditions more than the exotic species, Raghuvanshi (2001). George (2003) stated that even though the bulk of their weight is water, leafy vegetables represent a natural pharmacy of minerals, vitamins and phytochemicals. He concluded that the potassium content of leafy vegetables is good in the control of diuretic and hypertensive complications, because it lowers arterial blood pressure. The fiber content of vegetables contributes to the feeling of satisfaction and prevents constipation Noonan,(1999).

During host pathogens relationship, pathogens obtain their nutrients from host tissues and organs and a metabolic activities happens resulting in a spot or lesions on the leaf surface, called as symptoms. Symptoms vary on the basis of pathogen, host physiology and depends on interaction between them. Death of tissue express necrotic symptoms like browning, wilting die back, spotting, anthraconase (angular spots), mildew downy or powdery, rusts (Crust of iron). Atrophic symptoms (slowing down the development of affected parts) like dwarfism, chlorosis, and hypertrophic symptoms like curling appears on the leaves are externally examined by their color shape and sizes by naked eye. and internally examined by microscope of hand on microtome section.

Singh and Bhalla (2000) used techniques and mounted scrapings in lactophenol cotton blue in their microscopic studies on foliar diseases in plain forest of Vindhya region and subsequently found four new species of *Pseudocercospora* sps from fungal foliar lesions. The structure of perithecia and ascospores by microscope of different isolates of *Chaetomium globosum* studied by Khayum *et al* (2005). There exists a great degree of variations among isolates of any pathogen depending on geographical distribution. Prasad and coworkers (2008), researched on castor wilt caused by *Fusarium oxysporum sp. ricini,* isolates from various parts of India and found variation in mycelial growth, pigmentation, sporulation size of conidia and production of macro and microconidia. They also found variation among isolates with regards to production of toxin incidence. Thus stating that genetic differences attribute to pathogenic variability and disease morphology. Similarly,

variability for lesions size, lesion number of *Alternaria brassicae* in rapeseed mustard studied by Rajbeer yadav *et al* (2008).

Inoculation is the process when pathogen interacts with host resulting in development of disease.The role of infection and percentage of spore development affected by a complex of interesting factors including pH, temperature, moisture, susceptibility of the plant tissue and aggressiveness of the pathogen, Chandramohan *et al* (1987). Degree of sporulation affected by co factors temperature and pH, Patil and Rawat (1991), Prasad *et al* (1982), Arora (1984).

When a potential host come in contact with a pathogen, the substances produced by the pathogen make a harmful effect on the host. It may be sugar, aminoacid, organic acid or various types of extracellular or intracellular enzymes. The efficiency of sugar utilization is valuable in different organisms. Arora (1984), Singh *et al* (1987), reported that incidence of potato leaf spot caused by *Alternaria alternata* was significantly affected by date of planting, they also reported that with increase crop, as decrease in nitrogen content, in leaves to content along with increase in soluble sugar leading to susceptibility of mature plants to pathogens. The capacity of protein production is different in various organisms, Soni (1989), Ghosh (1991), Singh and Shukla (1987), studied amino acid changes in black gram leaves infected by *Colletotrichum truncatum* and estimation of amino acid from *Alternaria alternata* has done by Prasad and Reddy (1987).

Enzymes are biocatalysts and play very important role during pathogenesis. The enzymes produced by result of host pathogen interactions, some of them of extracellular in nature like pectolytic, cellulolytic, amylolytic and proteolytic some enzymes are intracellular in nature like phosphatase.

Gogoi *et al,* (2000) reported change in trends with respect to time after inoculation in two defence related enzymes viz., polyphenol peroxidase and phenylalanine ammonialyase in wheat when infected with *Neovossia indica*. Shantha and coworkers (1987) reported that the activity of enzyme cellulase in pathogenesis influenced by various amino acids.

Production of acid and alkaline phosphatase by pathogens which participate in the energy metabolism organisms, have been found by Sakurai *et al* , Arora *et al* (1983).

Foliar disease of foliaceous leafy vegetables decreases the quality and quantity of production of agricultural crops. Losses can be reduced by an economic method and effective source like antibiotics, fungicides and angiospermic plant products. Several methods for control of pathogenic fungi have been advocated by extensive use of chemical fungicides but recurrent and indiscriminate use of chemical fungicides have been proved to

be threat to human health hence use of biological control for controlling pathogenic fungi has been gaining popularity.

Kamil and coworkers (2009) experimented on effect of dominant fungi in green manure and found that cultural filtrates and volatile metabolites of *Trichoderma harzianum* in green manure significantly reduced mean colonial diameter of *Sclerotium rolfsii* and *Rhizoctonia solani*. Extracts of many plants have shown significant effect on spore germination of pathogenic and non pathogenic fungi cited by Singh *et al*, (1989). Singh and others (1991), found that extracts of ginger rhizome played a significant role in control of powdery mildew in pea plants. Similarly Chandrasekar and coworkers (1993) demonstrated a significant negative effect of foliar spray of potash and neem seed extract on tikka leaf spot of groundnut. Anti fungal activity of Azadirachtin against the growth of *Macrophomina phaseolina* by poisoned food technique by Dubey *et al* (2003). Extracts of four plants viz *Chromolaena odorata, Ageratum houstorium ,Polygonum hydropiper* and *Tagetus erecta* applied against *Colletotrichum capsici* on piper Utpal *et al* (2008), Methanol extract from medicinal plants like *Eucalyptus, Calotropis, Parthenium* were evaluated *against Alternaria blight* of mustard studied by Patni and coworkers (2005). Aqueous extract of *Allium cepa* on leaves of *Brassica juncea* against *Alternaria, Meena* and Chatopadhyay (2008).

Objectives

I. The diseased leaves of the selected vegetables will be analysed on the basis of

a. symptoms and morphological variations
b. inoculation
c. pathogenesis
d. control measures for their phytopathological studies

II. Isolated pathogens will be examined for

a. Sugar biomass
b. Protein
c. Organic acid
d. To elaborate the enzyme, protease, pectolytic and cellulolytic enzymes, acid and alkaline phosphatase.

III. *In vitro* and *in vivo* control measures.

The collected data will be used for interpretation of the pathogenic abilities of the pathogens.

Noteworthy contribution in the field of proposed work_

Bharathi (2011) stated that leafy greens are rich in calcium, vitamin A,B,C,K magnesium & iron. The green leafy vegetables contain a high concentration of folic acid which is good for pregnant women. Phytochemicals are an important component in most of the vegetables which have been cited as having extreme beneficial effect on women health while the antioxidant properties of certain phytochemicals have been scientifically proved Rechnar *et al* (2002).

Similarly different phytochemicals obtain from leafy vegetables studied like in *Rumex vesicaris* by Panduraju et al (2009), In *Leucas cephlotes* by Selva Narayana (1995), Chauhan (1999), Saudane *et al* (2000), Shukla (2010), Hemandra and coworkers (2011), Mathur *et al* (2011),*Ipomoea aquatica* was studied by Vimal Kumar *et al* (2009), in *Cassia tora* it is reported by Prabhu and Krishnamurthy (2011), , for *Oxalis corniculata* it is studied by, Sharan Gauda and Patil (2007), Mizokama *et al* (2008).

Vishwakarma and Dubey (2011) worked on bhajjis prevalent in Chhattisgarh and recommended use of these leafy vegetables to overcome the severe problem of malnourishment in the state. They worked on 25 types of leafy vegetables and worked out their nutritional contents by using proximate analysis.

Pathogenicity of a plant pathogens refers to its disease causing ability and various other factors including host pathogen interactions. During disease development pathogenic agent utilize nutrients according their efficiency and lower the nutritional ability and germination power of the host, Goswami *et al* (1996), Chaudhary *et al* (1984), Maheshwary *et al* (1984), Nair (1994), Bindu (1997). Sharma and Co worskers (2008), Kulkarni *et al* (2007), Gopinath and Shetty (1987).

. Isolated pathogenic fungi show a lot of variation with respect to morphology, pathology, physiology and molecular variability within same population of fungi. This distinct polymorphism was noted significantly in *Alternaria solani* isolates on tomato by Naik *et al* (2010). This polymorphism was seen in morphological variability as measured by length, breadth and beak size of conidia; variability in cultural characteristics like colony diameter and colony colour. They also noted that variants within population may affect the rate of disease development and induce infection in more host lines which might affect cultivar resistance.

Pandey (2009) studied on role of inoculum density and incubation period on saprophytic behavior of *Rhizobium sps.* Michael Davis (2006) found that holdover inoculum of *Pseudomonas syringe pv alisalensis* from broccoli raab caused disease in subsequent plantings. Severe infection of downy mildew was observed in 7 days old seedlings studied

by Rathore *et al* (1987).The development behavior like influence of tuber infection, wetting, duration incubation period, crop age, date of planting on late light studied by Singh *et al* (1987), Thind and Mohan (2008).

When a potential host come in contact with a pathogen, the substances produced by the pathogen make a harmful effect on the host. It may be sugar, aminoacid, organic acid or various types of extracellular or intracellular enzymes. The efficiency of sugar utilization is valuable in different organisms. Arora (1984), Karkashe (1984), Nair (1994).

When facing on slought of various diseases the enzyme profile of plants undergo changes. Sarkar and coworkers (2010), recorded increase in oxidative enzymes in banana varieties under pathogenesis of fruit rot fungi Suppression of essential pectolytic and cellulolytic enzymes during pathogenesis by sugar in the mechanism of disease resistance reported by Chakroborty *et al* (2005), Usha Malini *et al* (1998).

The natural substances of some plant species have been reported to posses toxic effect on many fungal pathogens which causes diseases on plants, Singh *et a*l (1983), Dubey (1991). Antifungal property of medicinal plant Garlic has been tested, Bisht and Khulbe (1995), *Ocimum sanctum* leaves, Tiwari and Nayak (1991), Ansari (1995) Scaly leaves of *Allium cepa*, Kulshrestha (1984), Nair (1994), *Trachispermum ammi* Ansari (1995).

Proposed methodology

I. SURVEY,SCREENING AND ISOLATION

1. A survey will be made to assess the sustainable use and ethnobotanical significance of the selected crops
2. The selected crop hosts will be collected from different fields in the vicinity of Raipur district duringdifferent crop seasons.
3. The morphological characterization of disease symptoms will be done.
4. Isolation - The fungal organasims from the disease symptoms will be isolated using standard techniques and their pure cultures will be maintained for further studies.

II. HOST PARASITE RELATIONSHIPS

To prove the pathogenicity, Koch's postulates will be applied.

III. Nutritional evaluation by using standard method.

IV. BIOCHEMICAL CHARACTERSTICS OF THE FUNGAL PATHOGEN

1. Sugar Biomass ratio
2. Protein Test,(Lowry *et al* 1951)

V. CHROMATOGRAPHIC ANALYSIS

1. Amino acids
2. Organic acids

VI. ENZYMOLOGICAL STUDIES

1. Extracellular enzymes
   a) Pectinase, polymethygalcturonase(PMG), polygalacturonase (PG), by usual method (Hancock *et al*,1964, Bateman,1963).
   b) Cellulase , (Gascoigne and Gascoigne 1960).
2. Intracellular
   a) Acid phosphatase
   b) Alkaline phosphatase

VII. DISEASE PROTECTION

Control Experiments (*in vivo and in vitro)*

a) Antibiotics
b) Fungicides
c) Angiospermic sources

Expected outcome of the proposed work

The outcome of this research work would be an attempt to understand

1. The biological totality of pathogenic manifestation of certain foliar fungal pathogens of selected foliaceous vegetables crops.
2. In these studies the isolated pathogens examined on the basis of biochemical and physiological aspects to understand on host pathogen interactions and disease manifestation.
3. The statistical data interpret the pathogenic abilities of the selected pathogens.

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